ORIGINAL ARTICLE

Analysis of Polymorphisms in T_H2-Associated Genes in Russian Patients With Atopic Bronchial Asthma

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Abstract

Background: Bronchial asthma is a chronic respiratory disorder characterized by airway inflammation, airway hyperresponsiveness, and periodic reversible airway obstruction. Subtype 2 helper T cell (T_H2) cytokines play an important role in the development of allergic airway inflammation in patients with bronchial asthma.
Objective: To investigate whether the single-nucleotide polymorphisms (SNPs) Ile75Val and Gln576Arg in the *IL4RA* gene, –33C>T in the *IL4* gene, and Gly237Glu in the *FCER1B* gene contribute to the development and severity of atopic bronchial asthma in Russian patients from Moscow.
Methods: We analyzed DNA samples from 224 patients with atopic bronchial asthma and 172 healthy individuals. Genotyping was performed by primer extension followed by matrix-assisted laser desorption/ionization–time of flight mass spectrometry.
Results: We observed a moderate association between the Arg/Arg genotype of Gln576Arg and protection against asthma (odds ratio [OR], 0.16; $P<.012$) and a strong association between the T allele and TT genotype of –33C> and atopic bronchial asthma (OR, 1.91 and 4.65, respectively; $P<.0001$). Carriers of the C allele had a reduced risk of asthma (OR, 0.53; $P<.0001$). Furthermore, we found that the TT genotype of –33C>T correlated with higher concentrations of total serum immunoglobulin E and interleukin 4 than the CC and CT genotypes.
Conclusion: We found an association between atopic bronchial asthma and the SNPs Gln576Arg in IL4RA and –33C>T in *IL4*. *IL4RA* and *IL4* seem to be involved in the pathogenesis of asthma.

Key words: Atopic bronchial asthma. SNP. Association. *IL4RA*. *IL4*. *FCER1B*.

Resumen

Antecedentes: El asma bronquial es un trastorno respiratorio crónico que se caracteriza por la inflamación, hiperreactividad y obstrucción reversible periódica de las vías respiratorias. Las citocinas producidas por los linfocitos T cooperadores de subtipo 2 (T_H2) desempeñan un papel importante en el desarrollo de la inflamación alérgica de las vías respiratorias en pacientes con asma bronquial.
Objetivo: Estudiar si los polimorfismos de un solo nucleótido (SNPs) Ile75Val y Gln576Arg en el gen *IL4RA*, –33C>T en el gen IL4 y Gly237Glu en el gen *FCER1B* contribuyen al desarrollo y a la gravedad del asma bronquial atópica en pacientes rusos de Moscú.
Métodos: Se analizaron muestras de ADN de 224 pacientes con asma bronquial atópica y de 172 individuos sanos. El genotipado se realizó mediante extensión del cebador seguida de espectrometría de masas MALDI-TOF.
Resultados: Se observó una asociación moderada entre el genotipo Arg/Arg de Gln576Arg y la protección frente al asma (oportunidad relativa [OR], 0,16; $p<0{,}012$), así como una estrecha relación entre el alelo T y el genotipo TT de –33C> y el asma bronquial atópica (OR, 1,91 y 4,65, respectivamente; $p<0{,}0001$). Los portadores del alelo C presentaron un menor riesgo de padecer asma (OR, 0,53; $p<0{,}0001$). Además, se observó que el genotipo TT de –33C>T estaba relacionado con concentraciones más elevadas de inmunoglobulina E sérica total y de interleucina 4 que los genotipos CC y CT.
Conclusión: Se halló una asociación entre el asma bronquial atópica y los SNP Gln576Arg en *IL4RA* y 33C>T en IL4. *IL4RA* e *IL4* parecen intervenir en la patogenia del asma.

Palabras clave: Asma bronquial atópica. SNP. Asociación. *IL4RA*. *IL4*. *FCER1B*.

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Introduction

The most common type of bronchial asthma, atopic bronchial asthma, is characterized by activation of subtype 2 helper T (T_H2) lymphocytes. Many genes participate in the pathogenesis of asthma, and susceptibility genes may differ according to population [1].

Interleukin (IL) 4 is a T_H2 cytokine that plays a central role in T-cell differentiation toward the T_H2 phenotype and in regulation of immunoglobulin (Ig) E production by isotype switching from IgM to IgE in B cells [2]. These 2 effects lead to the development of airway inflammation. IL-4 acts through the IL-4 receptor (IL-4R), which consists of 2 subunits, the α chain (IL-4Rα) and the γ chain (γc) [3]. IL-4 induces Ig heavy chain class switching to IgE by activation of germline Ig heavy chain transcription [4]. IgE subsequently binds to the IgE receptors FcεRI (high affinity) and FcεRII (low affinity), which are found on mast cells and basophils. IgE-dependent activation of mast cells and basophils through FcεRI is involved in the pathogenesis of the allergen-induced immune response in atopic diseases, including bronchial asthma. FcεRI consists of 3 subunits, namely, the α, ß, and γ chains. The β chain plays an important role in IgE-mediated allergic reactions [5].

The single-nucleotide polymorphisms (SNP) Ile75Val and Gln576Arg in *IL4RA*, –33C>T in *IL4*, and Gly237Glu in *FCER1B* have been associated with asthma [6-13]. However, many reports do not confirm this association [14-19]. Thus, the aim of our study was to assess the association between these polymorphisms and atopic bronchial asthma in Russian patients.

Materials and Methods

Participants

The study population comprised 224 unrelated patients with atopic bronchial asthma (cases) and 172 healthy individuals (controls) of Russian origin. The data collected were gender, age, age at onset of disease, asthma duration, asthma severity, smoking, antigen sensitization (domestic antigens [house dust mite, cat dander, and *Mucor*], pollen, food, drug), allergic comorbid conditions, and total serum IgE and IL-4 titers. The diagnosis of asthma was based on clinical history, physical examination, and spirometry (forced expiratory volume in the first second of expiration [FEV_1]). Atopy status was defined as follows: at least 1 positive skin prick test result with a common allergen; elevated circulating total IgE titers; and confirmed clinical symptoms of allergy. The characteristics of both groups are shown in Table 1.

Skin prick tests were performed to 6 common allergens: *Mucor*, tree pollen, grass pollen (Sevapharma a.s.), *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, and cat dander (Biomed). Positive controls (histamine 1 mg/mL) and negative controls (normal saline) were used. The wheal diameters were recorded after 15 minutes. A reaction ≥3 mm or ≥50% of the histamine response was accepted as positive (Table 1). Total serum IgE and IL-4 titers were measured using enzyme-linked immunosorbent assay (ELISA) test kits (IgE-ELISA and IL4-ELISA, CCPP).

We also tested the association between genetic markers and asthma severity by dividing patients with atopic bronchia asthma into subgroups of 114 patients with moderate or severe persistent asthma and 110 patients with mild persistent asthma according to the criteria of the Global Initiative for Asthma (GINA) [20].

The local medical ethics committee approved the study, and all participants gave their informed consent.

Nomenclature and Genotyping

We determined the genotypes of Ile75Val (rs1805010, c223A>G) and Gln576Arg (rs1801275, c.1727A>G) in *IL4RA* (NC_000016.9), –33C>T (rs2070874) in *IL4* (NC_000005.9), and Gly237Glu (rs569108, c.710A>G) in *FCER1B* (NC_000011.9). Gene codes are given according to the National Center for Biotechnology Information. Amino acid changes are designated considering that the first methionine is amino acid number 1. The genetic positions of

Table 1. Skin Prick Test Results in Patients Atopic Bronchial Asthma and Clinical Characteristics of Patients and Controls[a]

Aeroallergens	Positive
Tree pollen	42.6%
Cat dander	34.7%
Dermatophagoides farinae	54.5%
Dermatophagoides pteronyssinus	54.4%
Mucor	4.9%
Grass pollen	38.6%

	Mild asthma (n=110)	Moderate/severe asthma (n=114)	Control group (n=172)
Gender, Male/Female	57/53	48/66	98/74
Age, y	32.7 (10.5)	38.3 (12.6)	36.9 (10.1)
Asthma duration, y	10.7 (7.4)	11.5 (7.5)	0
Total serum IgE, kU_A/L	210 (53-535)	252 (128-645)	45 (23-89)
Total serum IL-4, pg/mL	11 (8-48)	20 (12-77)	2 (1-5)
FEV_1	87.5 (82.1-93.3)	78.6 (68.7-84.2)	–

Abbreviations: FEV_1, forced expiratory volume in 1 second of expiration; Ig, immunoglobulin; IL, interleukin.
[a]Age and asthma duration are shown as mean (SD); IgE, IL-4, and FEV_1 are shown as median (IQR).

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SNPs are given according to Human Genome Variation Society recommendations.

Genomic DNA was obtained from peripheral blood leukocytes of all the participants using a standard proteinase K digestion and phenol/chloroform extraction method. SNP genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics) according to a previously described protocol [21].

We used the following primers for the polymerase chain reaction (PCR): Ile75Val, F – 5'-AGA-GTC-TGA-TGC-GGT-TCC-TG-3' and R – 5'-CGC-ACT-GAC-CAC-GTC-ATC-C-3'; Gln576Arg, F – 5'-CTC-CGC-CGA-AAT-GTC-CTC-3' and R – 5'-GCC-TTG-TAA-CCA-GCC-TCT-CCT-3'; –33C>T, F – 5'-GGC-CTC-ACC-TGA-TAC-GAC-C-3' and R – 5'-AGG-GGG-AAG-CAG-TTG-GG-3'; Gly237Glu, F – 5'-AGG-TCC-CAG-AGG-ATC-GTG-TTT-ATG-3' and R – 5'-CCT-TGG-CTG-TGA-ATC-AGA-GTG-T-3'.

For the primer extension reaction, we used the following primers containing biotin label (bio) at the 5'-end and a photocleavable linker (L) at the eighth or ninth position to the 3'-end: Ile75Val, 5'-bio-GCC-TCC-GTT-GTT-CLT-CAG-GGA-3'; Gln576Arg, 5'-bio-GCC-CCC-ACC-AGL-TGG-CTA-TC-3'; –33C>T, 5'-bio-GTC-GAT-TTG-CAG-TGA-CLA-ATG-TGA-G-3'; Gly237Glu, 5'-bio-TGA-GTT-GGA-AGA-CLC-CAG-GGG-3'.

Statistical Analysis

Genotype and allele frequencies in case and control groups and in mild or severe asthma groups were compared using the Fisher exact test calculator for a 2×2 contingency table (http://research.microsoft.com/en-us/um/redmond/projects/MSCompBio/FisherExactTest). The difference between groups was considered statistically significant when $P<.05$. The odds ratios (OR) and 95%CI were determined to assess the relationship between the allele and genotype and bronchial asthma and were calculated using the calculator for confidence intervals of the OR (http://www.hutchon.net/ConfidOR.htm). Haplotype analysis was performed using an expectation-maximization algorithm (HAPSTAT). Hardy-Weinberg equilibrium was tested using a Hardy-Weinberg equilibrium calculator (http://www.oege.org/software/hwe-mr-calc.html) [22]. The association between genetic markers and clinical markers of atopy, such as total serum IgE and IL-4, were analyzed using the Mann-Whitney and Kruskal-Wallis tests (Statistica 6.0, Statsoft).

Results

Association With Atopic Bronchial Asthma and Asthma Severity

Before performing the association analysis, all the SNPs were tested for Hardy-Weinberg equilibrium in both groups.

Table 2. Distribution of Allele and Genotype Frequencies in Patients With Atopic Bronchial Asthma and Healthy Controls

Alleles and Genotypes	Frequency		*P*	OR (95% CI)	Frequency		*P*
	BA+	BA–			MA	SA	
			Ile75Val				
Ile	0.618	0.640	>.05	–	0.645	0.592	>.05
Val	0.382	0.360		–	0.355	0.408	
Ile/Ile	0.348	0.366	>.05	–	0.373	0.325	>.05
Ile/Val	0.540	0.547	>.05	–	0.545	0.535	>.05
Val/Val	0.112	0.087	>.05	–	0.082	0.140	>.05
			Gln576Arg				
Gln	0.830	0.791	>.05	–	0.832	0.829	>.05
Arg	0.170	0.209		–	0.168	0.171	
Gln/Gln	0.670	0.634	>.05	–	0.664	0.675	>.05
Gln/Arg	0.321	0.314	>.05	–	0.336	0.307	>.05
Arg/Arg	0.009	0.052	.012	0.16 (0.04-0.77)	0.000	0.018	>.05
			–33C>T				
C	0.623	0.759	4.484 10–5	0.53 (0.38-0.72)	0.659	0.588	>.05
T	0.377	0.241		1.91 (1.39-2.60)	0.341	0.412	
CC	0.487	0.581	>.05	-	0.527	0.447	>.05
CT	0.272	0.355	>.05	-	0.264	0.281	>.05
TT	0.241	0.064	1.164 10–6	4.65 (2.35-9.21)	0.209	0.272	>.05
			Glu237Gly				
Glu	0.984	0.994	>.05	–	0.986	0.982	>.05
Gly	0.016	0.006		–	0.014	0.018	
Glu/Glu0.969	0.988	>.05	–	0.973	0.965	>.05	
Glu/Gly0.031	0.012		–	0.027	0.035		

Abbreviations: BA, bronchial asthma; MA, moderate asthma; SA, severe asthma.

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Table 3. Distribution of Haplotype Frequencies of *IL4RA* Variants (Ile75Val and Gln576Arg) in Patients With Atopic Bronchial Asthma and Healthy Controls

Alleles and Genotypes	Frequency		*P*	Odds Ratio (95% CI)	Frequency		*P*
	BA+	BA–			MA	SA	
			Ile75Val				
Ile-Gln	0.543	0.518	>.05	–	0.571	0.517	>.05
Ile-Arg	0.076	0.121	>.05	–	0.075	0.075	>.05
Val-Gln	0.287	0.273	>.05	–	0.261	0.312	>.05
Val-Arg	0.094	0.088	>.05	–	0.093	0.096	>.05

Abbreviations: BA, bronchial asthma; MA, moderate asthma; SA, severe asthma.

Only –33C>T in the case group was not in Hardy-Weinberg equilibrium (*P*<.000001), but the other SNPs in both groups and –33C>T in the control group successfully passed the test (*P*>.05).

The genotype and allele distributions of all the SNPs studied are shown in Table 2. We found a direct association between –33C>T and atopic bronchial asthma. Carriers of the T allele and TT genotype had an increased risk of developing asthma (OR, 1.91 [95% CI, 1.39-2.60]; and OR, 4.65 [95% CI, 2.35-9.21, respectively; *P*<.0001). Individuals who carried the C allele had reduced susceptibility to atopic bronchial asthma (OR, 0.53; 95% CI, 0.38-0.72; *P*<.0001).

For Gln576Arg in *IL4RA*, a statistically significant difference in the distribution of the Arg/Arg genotype was observed in patients with atopic bronchial asthma compared with healthy controls (0.9% vs 5.2%). Carriers of the Arg/Arg genotype had a reduced risk of developing asthma (OR, 0.16; 95%CI, 0.04–0.77; *P*<.012). Unlike –33C>T, Gln576Arg did not demonstrate an association with increased risk of asthma.

No significant differences were found in the allele and genotype frequencies of Ile75Val and Gly237Glu between the groups. Furthermore, we did not detect any association between the SNPs tested and disease severity (Table 2).

Haplotype analysis was performed for Ile75Val and Gln576Arg. Haplotype frequencies are summarized in Table 3. No association was found between haplotype and atopic bronchial asthma or between haplotype and disease severity.

Association With Inflammation Markers

The concentration of clinical markers of atopy (IgE and IL-4) in the blood of asthma patients is an important factor in disease severity. We observed a relationship between total

Table 4. Distribution of Total Serum Immunoglobulin E and Interleukin 4 Concentrations Between Genotype Groups of Ile75Val and Gln576Arg in *IL4RA*, –33C>T in *IL4*, and Glu237Gly in *FCER1B* in Patients With Atopic Bronchial Asthma[a]

	Immunoglobulin E, kU_A/L		Interleukin 4, pg/mL	
	Median (IQR)	*P*	Median (IQR)	*P*
		Ile75Val		
Ile/Ile	250 (65-580)	>.05	12 (6-29)	>.05
Ile/Val	197.5 (65-700)		9 (3-52)	
Val/Val	700 (180-1500)		31 (6-103)	
		Gln576Arg		
Gln/Gln	235 (77.5-696.5)	>0,05	9 (3-52)	>.05
Gln/Arg	220 (45-700)		8.5 (3-77.5)	
Arg/Arg	550 (450-650)		35.5 (26-45)	
		–33C>T		
CC	90 (40-270)	<.0001	8 (3-14)	.003
CT	230 (80-580)		9 (3-23)	
TT	615 (227.5-1072.5)		25.5 (9-284)	
		Glu237Gly		
Glu/Glu	230(69-650)	>.05	11 (6-52)	>.05
Glu/Gly	1000 (200-1400)		5.5 (2-15)	

[a]Results are shown as median (IQR).

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serum IgE and IL-4 titers and the genotypes of each SNP (Table 4).

Significant differences between genotypes of –33C>T in IL4 were achieved for total serum IgE and IL-4 (*P*<.0001 and *P*=.003, respectively, Kruskal-Wallis test). Levels of total IgE and IL-4 in asthmatics with the TT genotype were 615 (227.5-1072.5) kU_A/L and 25.5 (9-284) pg/mL, respectively, being significantly higher than 90 (40-270) kU_A/L and 8 (3-14) pg/mL in asthmatics with the CC genotype (*P*<.0001 and *P*=.002, respectively, Mann-Whitney test) and 230 (80-580) kU_A/L and 9 (3-23) pg/mL in patients with the CT genotype (*P*=.004 and *P*=.008, respectively, Mann-Whitney test). Thus, the TT genotype of –33C>T correlated with higher concentrations of both atopic markers than the CC and CT genotypes.

No associations were found between the 3 SNPs and total serum IgE and IL-4 levels in patients with atopic bronchial asthma (*P*>.05).

Discussion

Binding of IL-4 to its receptor leads to the activation of Janus kinase family tyrosine kinases and subsequent activation of signal transducer and activator of transcription 6 (STAT6). Several findings suppose that any dysregulation in IL-4R/STAT6 signaling that results in enhanced STAT6 activity may cause increased T_H2 differentiation and predisposition to developing atopic disease [23].

Ile75Val in *IL4RA* is the isoleucine to valine substitution in the extracellular loop of the receptor. Ile75Val allelic frequencies vary widely according to population. Minor Val allele frequency was 36% in healthy Russian individuals, 45%-48.4% in European populations [15,24], and 52.4%-59% in Asian populations [6,9,25].

The findings of previous association studies of Ile75Val are inconsistent. An association was observed between the Ile/Ile genotype and atopic bronchial asthma in a Japanese population; in the same study, Ile75Val upregulated IgE synthesis and the receptor response to IL-4 [6]. However, Tan et al [7] showed that the Val allele was associated with atopy in Singaporean Indians. In the present study, we found no association between Ile75Val and atopic bronchial asthma. Our results confirmed previous reports in English, American, and Chinese adults [14,15,25] and in Mexican and Korean children [9,26]. A transmission disequilibrium test revealed neither linkage nor association between Ile75Val and atopy or asthma [27,28]. This mutation probably has very diverse effects on the development of asthma because of large allele frequency differences between populations. We did not find an association between Ile75Val and bronchial asthma, disease severity, or serum levels of total IgE and IL-4. Consequently, this polymorphism does not appear to play an important role in predisposition to asthma in Russians.

Another polymorphism in *IL4RA*, Gln576Arg, involves a glutamine to arginine substitution in the cytoplasmic domain of the receptor close to significant substrate binding sites for STAT6 and the phosphatase SHP-1. This substitution introduces an additional positive charge and probably alters the binding site for these substrates [29]. The minor Arg allele was found in 20.9% of healthy individuals of Russian origin; this figure is similar to that recorded for European populations, 21%-22% [17,29]. Frequencies are lower in Asian populations (11%-16%) [6,8,9].

The role of Gln576Arg in the pathogenesis of bronchial asthma is also controversial. Several reports showed no association between Gln576Arg and bronchial asthma in various ethnic populations [6,14,15]. However, Zhang et al [8] found an association between bronchial asthma and the Arg allele and Gln/Arg genotype in Chinese children, and Lee et al [9] observed an association between atopic bronchial asthma and the combined Gln/Arg+Arg/Arg genotype in Korean children.

In contrast, Kruse et al [29] reported a significant association between the Arg allele and low total IgE levels and demonstrated that this allele acts synergistically with the Pro allele of another SNP in *IL4RA* (Ser503Pro) to inhibit the signal transduction pathways of *IL4RA*. These findings were in direct contrast to the concept of the upregulatory effect of the Arg allele. This inconsistency may be due to increased phosphorylation of the insulin receptor–like substrates IRS-1 and IRS-2 in individuals with the Arg allele. This process possibly eliminates *IL4RA* signal propagation and has a negative effect on B-cell proliferation and IgE levels. We found that Russian patients carrying the Arg/Arg genotype had reduced susceptibility to developing atopic bronchial asthma, thus confirming the hypothesis of the negative effect of the Arg allele on the *IL4*/*IL4RA* signal transduction pathway. Of note, the effect of the Arg allele in the present study was moderate and was not associated with levels of markers of atopy.

The –33C>T polymorphism in *IL4* is a C to T substitution in the promoter of the gene close to the transcription initiation site, and this exchange was found to affect the promoter activity of the gene [16]. We identified the minor T allele in 24.1% of healthy Russians. This frequency was 14%-23% in Europeans [15,17], 28% in Iranians [11], and 67.1% in Japanese [16].

Several studies were unable to find an association between –33C>T and bronchial asthma [15-17]. However, in 2 reports, this association was demonstrated, and T allele carriers had an increased risk of asthma [11,30]. We also found a strong association between –33C>T and atopic bronchial asthma and observed that the TT genotype correlated with elevated concentrations of total serum IgE and IL-4 levels in asthmatic patients. These results may be explained by correlation of promoter polymorphisms with enhanced IL-4 activity [31]. More than 10 regulatory sites have been identified in the IL-4 proximal promoter region in humans. Gervaziev et al [32] showed that a promoter segment located 35 bp downstream of the transcription start site and 33 bp upstream of the first ATG codon of the translated sequence contained a polymorphic transcription factor binding site. Superior binding affinity was observed for the C allele. The major protein that binds to that site is transcription factor Oct-1. The possibility that octamer binding sites are inhibitors of transcription assemblies on the promoters of certain cytokine genes could explain enhanced transcription activity and the effects of the T allele.

Glu237Gly is a glutamic acid to glycine substitution in the cytoplasmic tail of the protein. In our study, 6% of healthy Russian individuals had the Gly allele. This frequency varies from 2.6% to 18% in different populations [8,12,13,19].

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The Gly allele is associated with atopic bronchial asthma and elevated total serum IgE levels [8,12,13]. Nevertheless, several studies failed to confirm a role of the Glu237Gly SNP in genetic predisposition for atopic asthma [18,19]. We did not find an association between this SNP and asthma. The functional significance of Glu237Gly is not yet known, although the Glu237Gly amino acid substitution in the cytoplasmic domain of *FCER1B* probably does not alter the intracellular signaling pathways in IgE-dependent activation of mast cells and basophils.

In conclusion, we identified a moderate association between Gln576Arg of *IL4RA* and decreased risk of atopic bronchial asthma and a strong association between –33C>T of *IL4* and increased levels of markers of atopy and increased risk of asthma in Russians. These findings are consistent with those of previous studies that demonstrated a role for these genes in asthma susceptibility. However, our results seem to contradict findings by other authors, thus demonstrating the difficulties in interpreting an association between polymorphisms and disease phenotypes. The effect of various patient inclusion/exclusion criteria and population-related factors could be responsible for these conflicting results. Thus, in order to determine which polymorphisms are really associated with asthma development or whether polymorphisms are in high linkage disequilibrium with asthma-associated SNPs, it is necessary to carry out comprehensive studies of extended haplotype blocks across candidate genes in large well-defined populations in combination with functional analysis of gene polymorphisms and proteins.

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